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Epigenetic Mechanisms in Repeat Expansion Disorders

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Abstract

Over the past 20 years, nucleotide repeat expansion disorders have informed our broader understanding of neurodevelopmental and neurodegenerative disease. One area where this is especially true is the contributions of epigenetic mechanisms to neurological disease pathogenesis. This review describes a few of the myriad ways in which epigenetic processes underlie aspects of repeat expansion disorder pathophysiology and discusses how therapies targeted at epigenetic modulation hold promise for many of these disorders.

Keywords

Fragile X Syndrome; Myotonic Dystrophy; Spinocerebellar Ataxia; Huntington Disease; neurodegeneration; HDAC inhibitors

Introduction

Nucleotide repeat expansion disorders comprise a heterogeneous group of diseases that result from instability and expansion of simple tandem repeats (usually tri-nucleotide repeats). Pathogenic expansions can occur in coding or non-coding regions of genes. In disorders such as Friedreich Ataxia, expansions in non-coding regions cause transcriptional silencing or down-regulation of the associated gene and therefore act as recessively inherited, loss-of-function mutations[1–2]. In contrast, in disorders such as Huntington disease, tri-nucleotide expansions in the protein coding region introduce an abnormally long stretch of a single amino acid (often glutamine) into the associated protein which leads to a dominantly inherited, gain-of function mutation[3]. In the nine known polyglutamine diseases, the mutant proteins accumulate in ubiquitin-positive inclusions and interfere with cellular homeostasis through several different mechanisms (for recent reviews, see [3–4]). A third set repeat expansion disorders, typified by myotonic dystrophy (DM1), result from dominantly inherited noncoding repeat expansions that elicit toxicity via a gain of function mechanism as RNA[5–6].

Intensive research on these relatively rare disorders have often served as stepping stones to greater understanding of basic biological processes, including learning and memory, protein quality control, RNA processing, and neurodegeneration. Among the fields which have informed and been informed by work on these diseases is epigenetics. In almost all repeat expansion disorders described to date, a role for epigenetic alterations in pathogenesis have been proposed (see Tables 1 and 2), either as a mechanism to explain intergenerational or somatic repeat instability, as an explanation for silenced or elevated expression of the mRNA in which the repeat resides, or in the case of the polyglutamine diseases, a direct role

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for the toxic gain of function protein in epigenetic and transcriptional regulation. This review is designed to give an overview of how epigenetics informs our understanding of these disorders and provides a few specific examples that are covered in detail. By its very nature, it is not comprehensive; we thus refer the reviewer to more specialized reviews on this material for further details[1,4–9]. We also apologize to those colleagues whose outstanding work we excluded due to space limitations.

An Epigenetic Primer

Epigenetics can be broadly defined as any potentially heritable modification that alters gene expression without resulting from direct changes in the primary DNA sequence. Historically, the concept of epigenetics was proposed as a mechanism to explain how a single pluripotent cell with presumably a single genome could give rise to multiple different cell types with different morphologies and behaviors[10]. Although epigenetic changes by definition are potentially heritable, either through serial cellular divisions or across generations, the definition has morphed over time to include changes which are often transient and modifiable within a given cell. For example, numerous epigenetic alterations occur in terminally differentiated neurons in aging, neurodegeneration and learning and memory consolidation[9,11–13].

The majority of epigenetic regulation can be broken down into one of three basic mechanisms: nucleotide modification, such as methylation and hydroxymethylation of cytosine; histone modification, and nucleosome positioning. These mechanisms interact to determine the relative accessibility of a given genetic locus to activating and suppressing transcription factors. However, manipulation of any single one of these mechanisms can have broad and often detrimental effects on genome-wide transcriptional regulation.

Nucleotide modification, most commonly cytosine methylation, has been studied extensively in the context of epigenetic silencing associated with tissue differentiation, genetic imprinting, and X-inactivation[14–17]. This methylation most commonly occurs at CpG dinucleotide rich genomic regions known as CpG islands located in gene promoters[18]. When unmethylated, these regions typically maintain a more open chromatin structure allowing transcriptional activation[18–19]. Methylation of CpG islands and neighboring GC rich promoter regions can accompany or trigger heterochromatin formation[18–19]. DNA methylation induced transcriptional silencing can occur by recruitment of methyl CpG binding domain (MBD) proteins that provide a scaffold for silencing chromatin remodeling complexes[9]. Alternatively, methylated DNA can directly inhibit interactions between DNA binding proteins and their targets sequences within promoters[20].

Histones also play a major role in epigenetics. The core histones H2A, H2B, H3 and H4 together form the nucleosome, the major unit for DNA organization. All histones are subject to post-transcriptional modifications, including acetylation, methylation, phosphorylation, ubiquitination, and SUMOylation and these various modifications have important roles in transcriptional regulation, DNA repair and replication, and chromosome condensation[9]. The exact effects of all potential combinations of post-translational modification on the activity of DNA associated with a particular histone are complicated and still being worked out, but some general rules apply[9]. For example, acetylation of certain residues, especially on histone H3 and H4, and trimethylation at H3K4 in particular are associated with euchromatin and transcriptional activation, whereas deacetylation and methylation at H3K9 and H3K27 are often associated with heterochromatin and transcriptional silencing[9]. Acetylation and deacetylation of histones are mediated by histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. Each of these enzyme families has

numerous subclasses with differential activities at both specific histone residues and on the regulation of specific genes and processes.

In addition to the modifications discussed above, the position of nucleosomes relative to the start sites of genes is an important regulator of transcription. Most active genes maintain a nucleosome free region just upstream of the transcription start site, allowing access by the transcriptional machinery[21]. However, for some genes, nucleosomes can act as a barrier to transcription that must be displaced for gene activation to occur. Nucleosome positioning is significantly influenced by both DNA methylation as well as histone modifications[9].

Effects of repeat expansion in *cis* on chromatin structure and transcriptional regulation

Nucleotide repeat expansion can alter local chromatin structure through a number of mechanisms. *In vitro,* pure CAG/CTG repeats elicit strong nucleosome positioning signals whereas unmethylated CGG/CCG repeats have the opposite effect[22–23]. *In vivo*, their effects are more complicated and influenced by sequences surrounding the repeat and the methylation status of cytosines within the repeat itself. In a number of diseases, it is clear that transcription of repeat disorder containing transcripts is negatively influenced by both the repeat and the epigenetic context in which that repeat resides (see table 1). We focus here on the Fragile X spectrum disorders FXTAS and Fragile X Syndrome. We also discuss how effects of repeats in *cis* might contribute to phenotypic variability in myotonic dystrophy.

Fragile X Spectrum Disorders: a case of epigenetic extremes

Fragile X Syndrome (FXS) is the most common known inherited cause of cognitive impairment and autism[24–25]. The name “fragile X” itself reflects its long history as a disorder with an aberrant chromatin signature. Originally, the constellation of symptoms and signs associated with this condition was called Martin-Bell syndrome after the clinicians who described it[26]. However, in the late 1960’s, it was recognized that lymphoblasts derived from these patients demonstrate a predictable “fragile site” on the long arm of Chromosome X at Q27.3, observed as an isochromatid gap in karyotype staining when cells are grown in culture under deoxynucleotide perturbing conditions [27–28]. 30 years later, the region coincident with this fragile site was found to contain a polymorphic CGG tri-nucleotide repeat expansion in the 5′UTR of a gene, *FMR1*[29–31]. In patients with fragile X syndrome, this repeat is greatly expanded, often to thousands of (CGG)s, leading to transcriptional silencing of the *FMR1* gene and absent expression of the Fragile X Mental Retardation Protein, FMRP[29,32].

Over time, it has become clear that the original Martin Bell Syndrome is but one of many phenotypes associated with expansion of this CGG repeat. Normally, this sequence is less than 45 CGG repeats. A “full mutation” expansion to greater than 200 CGG repeats usually leads to transcriptional silencing and FXS. By contrast, patients with more modest expansions to between 55 and 200 CGG repeats do not develop early cognitive impairment but are instead at risk for the late onset neurodegenerative disorder Fragile X-associated Tremor Ataxia Syndrome (FXTAS)[33]. This condition usually occurs in male maternal grandfathers of FXS children over the age of 50, with an age dependent penetrance of greater than 50% in men and 15% in women by the time they reach their 80s[34–35]. Typical features variably include a gait-predominant cerebellar ataxia, intention tremor, dementia, Parkinsonism, peripheral neuropathy and neuropsychiatric symptoms[36]. In contrast to full mutations, this “premutation” range repeat is transcribed efficiently, but the CGG repeat expansion induces significant translational inefficiency in the FMR1 mRNA, likely by

forming a hairpin secondary structure in the 5′UTR that impairs ribosomal scanning [37–38]. Thus levels of the Fragile X Mental Retardation Protein (FMRP) are lower in both FXTAS patients and in mouse models of the disease,, despite a 2–8 fold increase in basal FMR1 mRNA levels[39–41]. In addition to FXTAS, premutation repeats are associated with premature ovarian failure[42] and may also lead to an increased incidence of autistic spectrum disorders and neuropsychiatric disease[43].

The mechanism by which the *FMR1* gene is transcriptionally silenced in Fragile X Syndrome has been an area of significant research over the past 20 years[1]. The CGG repetitive element as well as an upstream CpG island in the *FMR1* promoter is abnormally hyper-methylated in most affected individuals[32,44–47]. Initially, this methylation was thought to be the primary mediator of epigenetic silencing, with secondary recruitment of histone deacetylases and methyltransferases driving formation of a heterochromatin region over the *FMR1* locus (Figure 1). Indeed, this DNA methylation pattern is associated with histone deacetylation and heterochromatin formation across the FMR1 gene in differentiated cells [48–50]. Subsequent work has also demonstrated specific histone methylation marks across the FMR1 promoter and first exon, including di- and tri-methylation at Histone H3K9, and trimethylation at H4K20 and H3K27[50–53]. In rare full mutation patients with no DNA methylation in either the repeat or the upstream promoter, transcription is preserved[53–54]. *In vitro*, treatment with the DNA demethylating agent 5-aza-2-deoxycytidine (5-azadC) partially reactivates transcription and leads to a reversal of many but not all chromatin changes associated with transcriptional silencing[52,55–56].

Until recently, evaluating the temporal processes involved in transcriptional silencing of full mutation *FMR1* loci has not been feasible. A significant advance in this area occurred with the use of human stem cells derived from a full mutation pre-implantation embryo. As undifferentiated human embryonic stem cells (hESCs), these cells demonstrate *elevated* FMR1 mRNA transcription compared to controls[57]. The *FMR1* locus is unmethylated and associated with acetylated histones, consistent with a euchromatin state despite the presence of between 200 and 1000 CGG repeats. However, upon differentiation, FMR1 transcription decreases significantly. Surprisingly, this transcriptional suppression precedes DNA methylation at the FMR1 locus, but histone acetylation at H3K9 is lost and replaced by histone methylation at that same lysine. Subsequent differentiation into fibroblasts triggers methylation and transcriptional silencing at this locus, suggesting that methylation is not the initial event driving heterochromatin formation [57–58]. Interestingly, re-derivation of induced pluripotent stem cells from these same fibroblasts fails to reactivate transcription or demethylation of the DNA at the repeat, suggesting that gene inactivation, once achieved, is a stable state[58].

An alternative model for *FMR1* gene silencing in FXS proposes a role for RNA induced transcriptional silencing (RITS)[1,59]. This process involves the formation of double stranded RNA and components of the RNA interference pathway[60–61]. RITS most often arises in the setting of bidirectional transcription, which occurs at the FMR1 5′UTR and promoter[59,62].. However, evidence to date for a role or RITS in FXS pathogenesis remains largely circumstantial and it is unclear why this process would not be triggered by shorter repeat sizes as occur in FXTAS. Thus, work in emerging model systems like hESCs is still needed to determine the exact mechanism by which transcriptional silencing occurs.

In contrast to FXS, premutation carriers express too much FMR1 mRNA, often demonstrating a 2–5 fold increase in expression with repeat sizes between 55 and 200. The repeat containing mRNA itself is thought to elicit neurodegeneration through a toxic gain of function mechanism[5,63–66], implying that increased FMR1 mRNA accumulation is a proximal step in disease pathogenesis. The increase in FMR1 mRNA is not the result of

increased RNA stability induced by the repeat but instead reflects increased transcription[67]. Our group evaluated the hypothesis that the elevation in FMR1 mRNA expression might reflect an intrinsic epigenetic response related to the CGG repeat expansion. In a *drosophila* model of FXTAS where the 5′UTR of the FMR1 gene is placed upstream of a reporter gene (eGFP), co-expression of any of three different classes of HDACs led to suppression of CGG repeat induced neurodegeneration and transcriptional silencing of the transgene[68]. This suppression requires the presence of the CGG repeat expansion. In FXTAS patient derived cell lines, there is an increase in acetylated histones at the FMR1 locus around the repeat that correlates with both CGG repeat length and FMR1 mRNA expression. Interestingly, both the elevated FMR1 mRNA expression and the histone acetylation could be reversed by treatment with histone acetyltransferase inhibitors, suggesting that FMR1 transcriptional upregulation may be dynamic and modifiable. Taken together with *in vitro* data that CGG repeats actively exclude nucleosome assembly[22], these findings suggest that unmethylated CGG repeat expansions act in *cis* to create a more open chromatin structure that favors increased transcription.

However, not all data to date is consistent with this hypothesis. Studies in Xenopus oocytes demonstrate transcriptional repression with moderate sized CGG repeat elements introduced into the 5′UTR of a heterologous gene that were partially reversed by treatment with HDAC inhibitors[69]. More recently, a group generated stable integrated cell lines with interrupted CGG repeats of different sizes[70]. These cell lines showed decreased transcription with increasing repeat size above 30 CGGs that were partially corrected by demethylating agents[70]. It is possible that the interruptions (a CTAGG every 20 CGGs) explain this observation as AGG interruptions *in vitro* alter the ability of CGG repeats to incorporate into nucleosomes[22]. Nevertheless, given data demonstrating transcriptional upregulation with expanded repeats in two different knock-in mouse models[40,66,71] and in human patient derived fibroblasts, lymphoblasts and brains, it appears likely that in the context of the native *FMR1* locus, larger uninterrupted and unmethylated CGG repeats favor an open chromatin state, as is often seen with large CpG islands through the genome.

Insulation, DNA methylation, and congenital Myotonic Dystrophy

Myotonic Dystrophy type I (DM1) is the third most common cause of muscular dystrophy[6]. Patients with the classical adult onset form of DM1 present with distal muscle weakness, myotonia and complications related to cardiac dysfunction, cataracts, and mild neuropsychiatric symptoms[6,72]. However, the range of potential phenotypes in DM1 is quite broad, from mildly affected patients who have only cataracts and mild myotonia to a congenital form of DM1 characterized by diffuse hypotonia, respiratory compromise, and cognitive impairment in over 50% of patients[6–7]. DM1 results from an expanded CTG repeat in the 3′UTR of the *DMPK* gene[73–75]. Normally, the repeat is up to 35 CTGs. Repeat sizes from 50–150 lead to only mild symptoms, while repeats between 100 and 1000 usually cause the classical phenotype. In patients with congenital DM1 (cDM1), the repeats are usually larger than 2000 CTGs, but can be in a range more typically associated with the classical phenotype. After initial evaluations ruled out a significant role for insufficiency of the DMPK protein or neighboring proteins in disease pathogenesis for at least the adult onset form of the disease[76–80], attention turned to how the CUG repeat as RNA might elicit toxicity via sequestration of specific RNA binding proteins[81–83]. Specifically, CUG repeats can bind to and sequester the RNA splicing factor MBNL, leading to altered splicing in *trans* of mRNAs important for muscle function and myotonia.

Perhaps the most compelling evidence for this hypothesis came with the identification of the gene responsible for myotonic dystrophy type 2 (DM2)[84]. DM2 has a clinical phenotype that is similar to DM1, but results from a CCUG expansion in an intronic region of an unrelated gene, zinc finger nuclease 9[84]. These CCUG repeats act very much like their CUG

counterparts in DM1, forming nuclear RNA foci that sequester MBNL proteins and are associated with similar splicing defects[84–85]. However, in DM2, there is no congenital form of the disease and only minimal central nervous system involvement despite very large (>10,000) CCUG repeat expansions. This discrepancy has led multiple investigators to re-analyze the DM1 locus and the epigenetic effects of large CTG repeat expansions.

The *DMPK* locus lies in a gene-enriched region of chromosome 19, where the 3′UTR of *DMPK* is contiguous with the promoter of a neighboring gene, *Six5.* The CTG repeat itself is surrounded by two CTCF binding sites[80]. CTCF is a multifunctional zinc finger protein that binds to DNA and influences gene expression, nucleosome positioning, and chromatin organization[86]. CTCF binding can serve as a genetic insulator, preventing co-activation or suppression of one gene with that of close neighbors[86]. At the DM1 locus, these CTCF sites prevent the co-activation of DMPK transcription with *Six5,* which encodes a homeodomain protein expressed during early development[80]. An additional layer of complexity arises with production of an antisense transcript that initiates within the *Six5* promoter and extends back through the DM1 repeat, producing a CAG-containing noncoding RNA[60]. This antisense transcript can trigger RNA induced transcriptional silencing (RITS) and heterochromatin formation around the repeat[60]. However, antisense transcription through the repeat and subsequent RITS is impeded when CTCF binding is present[60].

CTCF binding to DNA is methylation state sensitive, with decreased binding at methylated DNA. In DM1 the CTG repeat induces DNA methylation throughout the *DMPK* 3′UTR that can exclude CTCF binding[60,87]. In fetal derived patient samples, this methylation is most pronounced proximal to the repeat[87]. However, the correlation of these events to gene expression changes from the *DMPK* and *Six5* loci is imperfect and may vary through development[7,87]. Thus, large CTG expansions and their accompanying chromatin changes can potentially trigger three alternative pathogenic pathways[7]. First, they may lead to decreased *Six5* transcription during early development due to RITS. Alternatively, the loss of CTCF binding could allow temporally aberrant expression of large CUG containing mRNAs during early development when the Six5 gene is most active[7]. Lastly, the transcription of long CAG containing RNA may be toxic independent of DMPK expression[88]. To date, there is no direct evidence of these events during early development. However, new models such as hESC or iPS cells may soon reveal answers to these important questions[89–90].

Epigenetic and transcriptional dysregulation in polyglutamine disorders

An emerging concept in polyglutamine (PolyQ) disorder research is the role of alterations in native protein function in disease pathogenicity[91–92]. Although polyglutamine tracts expressed in isolation elicit neurodegeneration, the normal function of the host protein greatly influences the specificity and mechanism by which this neurodegeneration occurs. In this context, it is intriguing that all of the known polyglutamine proteins have direct or indirect roles in transcriptional and epigenetic regulation in their native contexts (Table 2)[8]. Moreover, in most if not all of these disorders, there is evidence for broad spectrum alterations in transcription that accompany and sometimes precede neurodegeneration[8]. We focus here on three examples where these effects have been extensively studied: SCA1, SCA7, and Huntington disease.

Spinocerebellar Ataxia Type I: Altered Ataxin I interactions drive disease pathogenesis

Ataxin-1 is the protein mutated in Spinocerebellar Ataxia type I, a common cause of dominantly inherited cerebellar degeneration. Ataxin-1 is normally a predominantly nuclear protein widely expressed in the central nervous system [93]. Studies of transgenic animal models of SCA1 reveal down regulation of numerous genes during early development of

cerebellar Purkinje cells [94–95]. Two transcription factors, the retinoid acid receptor-related orphan receptor alpha (RORα) and Capicua, play prominent roles in SCA1 pathogenesis [96–97]. Capicua (CIC), is a transcriptional repressor containing a sox-like high mobility group (HMG) box. Human Capicua forms complexes with Ataxin-1 and co-expression of Ataxin-1 synergistically enhances CIC based transcriptional suppression. However, this enhancement is absent with a mutant Axaxin-1 containing 82 polyQ repeats[96]. Interestingly, both the polyglutamine dependent impairment of transcriptional repression and neurodegeneration are abolished with a S776D point mutation distant from the repeat. This point mutation also alters the ability of Ataxin-1to interact with Capicua. Perhaps more impressive is that cerebellar neurodegeneration can be triggered in the *absence* of polyglutamine expansions by a phospho-mimetic mutation at this same residue, suggesting that the interactions influenced by the phosphorylation state of this residue are critical to disease pathogenesis[92,96].

Although SCA1 is an adult onset neurodegenerative disorder, abnormalities occur during cerebellar development in *SCA1*[82Q] transgenic mice. When the SCA1 mutant transgene is expressed from early developmental stages of the cerebellum (3 days postnatal), the mice are profoundly ataxic. In contrast, when the mutant transgene is turned on after cerebellar development is complete (12 weeks postnatal), no ataxic phenotype is observed[97]. The transcription factor RORα plays a critical role in the *SCA1*[82Q] mediated early cerebellar developmental defects. Ataxin-1 interacts with RORα and the Tat-interactive protein 60 kDa (Tip60), a histone acetyltransferase and a nuclear receptor coactivator[98]. Tip60 mediates the interaction between Ataxin-1 and RORα[99] and it directly interacts with Ataxin-1[97]. Under partial loss of Tip60 (*Tip60*$^{+/-}$ background), *RORα* expression is increased in *SCA1[82Q]* transgenic mice [100] and these mice show delayed disease progression, indicating Tip60 interaction with the mutant SCA1[82Q] accelerates pathogenesis, likely through epigenetic modifications at Tip60 targeted genes[99].

Spinocerebellar ataxia type 7: a direct role in epigenetic modulation

Spinocerebellar Ataxia type 7 (SCA7) is unique in the polyQ neurodegenerative diseases in that it elicits retinal degeneration[101]. Ataxin-7 interacts with the retina specific transcription activator Cone-rod homeobox protein (CRX)[102]. Both wildtype and mutant ataxin-7 bind to CRX, but mutant ataxin-7 sequesters CRX into nuclear aggregates, triggering disruption of CRX-mediated expression of retina specific genes[102]. However, in a knock-in mouse model of SCA7, decreases of other rod specific genes like Rom1 precede CRX mediated effects, indicating that the sequestration of CRX via mutant ataxin-7 is not the only pathway involved in retinal degeneration in this model[103].

How normal and mutant ataxin-7 influence gene transcription became clearer after the observation that ataxin-7 serves as the subunit of histone acetyltransferase GCN5 complex TFTC (the TATA-binding protein-free TBP associated factor-containing complex) and the STAGA complex (the SPT3/TAF GCN5 complex) [104]. STAGA/TFTC complexes are the mammalian homologue of yeast SAGA (Spt/Ada/Gcn5) complexes which acetylate nucleosomal histones and serve as a transcription coactivators[105]. The expansion of polyQ in ataxin-7 does not stop its incorporation into such complexes [104,106], but it does affect the composition of the STAGA complex in that some components such as ADA2b and Spt3 of STAGA are diminished [107–108]. Such alterations compromise their HAT activity. Although free histone acetylation *in vitro* is not altered by mutant ataxin-7, polyglutamine expansion of ataxin-7 substantially disrupts the acetylation of histones in nucleosomes[107]. In mouse retina, CRX recruits the STAGA complex via binding to ataxin-7 to the promoters of CRX-responsive genes, leading these promoters to have higher levels of H3 acetylation and gene transcription. In contrast, mutant ataxin-7 inhibits STAGA complex HAT activity in the retina at these same genes, leading to hypoacetylation of H3 histones associated with

promoters of CRX responsive genes[108]. Thus, in SCA7, the polyglutamine expansion leads to direct dysfunction in an epigenetic maintenance complex that plays a critical role in retinal degeneration in SCA-7.

Huntington Disease: Global transcriptional suppression and an altered epigenetic profile

Huntington disease (HD) is an autosomal dominant neurodegenerative disease due to polyQ expansion in the N-terminus of the huntingtin (HTT) protein[3]. HTT is a large (348Kd) predominantly cytoplasmic protein which contains multiple protein-protein interaction motifs and which interacts with a large number of neuronal proteins. While the mechanisms of disease pathogenesis in HD are myriad[3–4,109], one important pathway involves dysregulated gene expression through altered interactions of mutant HTT with specific transcriptional and epigenetic coactivators[8]. These interacting proteins include Sp1, REST, and CBP.

Sp1 is a transcriptional activator that binds to upstream GC-rich elements of target genes and recruits the general transcription factor TFIID[110]. The N-terminal fragment and full-length HTT both interact directly with Sp1[111–112]. Both *in vitro* experiments and in mouse models of HD, the expanded polyglutamine stretch in mutant HTT increases binding to Sp1[112]. This interaction inhibits binding of Sp1 to important target gene promoters, including the promoter of nerve growth factor receptor (NGFR)[112]. Huntingtin can also affect SP1 targets by binding to the TBP associated factor TAFII130, a coactivator of Sp1 and a component of TFIID[111]. Mutant huntingtin inhibits dopamine D2 receptor gene expression in cultured striatal cells and this inhibition is reversed by co-expression of either Sp1 or TAFII130[111]. Transcription dysregulation by mutant huntingtin via Sp1 was further validated through chromatin immunoprecipitation analysis of Sp1 target genes. Although Sp1 protein levels are normal in HD mouse and HD patient brains, Sp1 promoter binding activity at susceptible genes is decreased[113].

HTT also interacts directly with the repressor element 1 (RE1) silencing transcription factor/neuron restrictive silencer factor (REST/NRSF)[114]. REST/NRSF is a transcription factor that binds to neuron restrictive silencer elements (NRSEs). Initially it was thought that REST acted primarily to repress target gene expression in non-neural tissues[115–117]. However, a number of observations over the past decade now suggest it also plays prominent roles in neurons, where it can act as a regulator of chromatin organization at numerous genes via its interactions with numerous corepressor complexes[118]. Normally, HTT binds to and sequesters REST in the cytoplasm, allowing de-repression of NRSE containing genes in neurons. However, mutant huntingtin fails to adequately sequester REST, leading to aberrant nuclear accumulation of REST and repression of numerous target genes, such as brain-derived neurotrophic factor (BDNF)[114]. Consistent with this, higher REST occupancy is seen at NRSE containing promoters in both homozygotic HD knock-in mice and HD transgenic mice[119]. REST interacts with numerous epigenetic modifiers to achieve gene expression repression. Specifically, REST can associate with histone demethylases such as SMCX, which is involved in X linked mental retardation [120], Histone Deacetylases such as HDAC1 and 2, histone methyltransferases such as G9a [121], and the chromatin remodeling factor SWI/SNF [122]. Further study of the how those epigenetic modifiers participate in REST-associated transcriptional changes in HD may well produce specific therapeutic targets[109].

A more direct connection to dysregulation of the epigenetic landscape in HD comes from work on the CREB-binding protein (CBP). CBP, a histone actetyltransferase and transcription co-activator in CREB-mediated transcription [123–124], is found in polyQ aggregates of HD [125]. HTT interacts directly with CBP through a short PolyQ tract that is present normally in CBP, and mutant HTT sequesters CBP into aggregates, effectively

depleting it in HD neurons and triggering alterations in CREB mediated gene transcription[126–128]. Consistent with this observation, overexpression of CBP rescues the mutant HTT toxicity in cells and in *drosophila*[126–127]. The polyQ tracts in HTT can bind directly to the histone acetyltransferase domains of both CBP and p300/CEB-associated factor (P/CAF), interfering with their ability to add acetyl groups to histones[129]. Expression of the mutant N terminal of HTT in cultured cells decreases histone acetylation at H3 and H4, while HDAC inhibitors can reverse this reduction[129]. Hypoacetylation of H3 at down regulated genes is also observed in HD transgenic mice [130], and treatment of wildtype cells with specific histone acetyltransferase inhibitors decreases expression of these same target genes. These studies make a strong argument that altered histone acetylation, especially at CREB target genes, may play an important role in the progression of HD.

Epigenetic therapeutic targets in Repeat disorders

Given the epigenetic perturbations associated with repeat expansion disorders, it is perhaps not surprising that epigenetic therapeutic targets have been proposed for a number of these diseases. In Fragile X Syndrome, a number of techniques have been tried to achieve transcriptional reactivation. DNA demethylating agents such as 5-azadC partially reactivate transcription from the *FMR1* locus in FXS patient derived cells, but the agents have proven too toxic to consider using in patients and do not work in non-dividing cells[55]. In contrast, broad spectrum HDAC inhibitors such as Trichostatin A fail to significantly reactivate *FMR1* transcription[48,55–56]. However, a more recent study suggests that significant reactivation might be achieved by utilizing sirtuin subtype HDAC inhibitors[56]. Treatment with the SIRT1 specific inhibitor splitomycin was able to achieve gene reactivation and partial reversal of the heterochromatin state of the *FMR1* locus in patient derived cell lymphoblasts. Interestingly, this re-expression was achieved without a significant change in DNA methylation over the repeat or the upstream CpG island in the *FMR1* promoter[56]. However, in patients with very large CGG expansions (>500), this increase in FMR1 mRNA was not accompanied by any production of FMRP, likely because of translational block induced by the CGG repeat as mRNA[54]. Although the safety profile of this class of agents in humans remains to be determined, in FXS patients with shorter repeats that do not show complete translational inefficiency, such therapeutic approaches remain promising[56].

Friedreich Ataxia (FA) is an autosomal recessive disorder characterized by slowly progressive ataxia, dysarthria, weakness and cardiomyopathy[2]. FA typically results from GAA expansions in the first intron of *FXN,* a gene which encodes the mitochondrial protein Frataxin[2]. As with FXS, this repetitive element elicits heterochromatin formation over the *FXN* locus and transcriptional silencing in a length dependent manner. However, in contrast to FXS, some low level of *FXN* transcription and functional Frataxin is present in all FA patients, as its complete loss is lethal during early embryogenesis and small differences in mRNA expression impact both the age of onset and the severity of the disease[2,131–132]. Further, the GAA repeat remains unmethylated and is spliced out of the mature mRNA, meaning it has no impact on translational efficiency. Thus, in many ways, it represents an ideal target for epigenetic therapies. Indeed, treatment of FA patient derived cell lines with broad spectrum HDAC inhibitors lead to significant transcriptional reactivation[133]. Subsequent work has identified a specific class of pimelic o-aminobenzamide HDAC inhibitors that achieve gene reactivation in mouse models of the disease and demonstrate reversal of key pathological findings[134–136]. Phase one clinical trials with these agents are planned.

In Huntington disease, large scale transcriptional down regulation of numerous genes is an early event in pathogenesis[130,137]. Many of these transcriptional abnormalities are associated with epigenetic alterations that are broadly distributed across the genome.[130]

Consistent with this, in both *Drosophila* and mouse models of HD, broad spectrum HDAC inhibitors suppress neuronal degeneration and reduce lethality[129,138]. Attempts to narrow the spectrum of such agents in a *drosophila* disease model suggest that inhibition of sir2 (akin to the mammalian HDAC SIRT1) and RPD3 (akin to class I HDACs in mammals) are synergistically beneficial in suppressing toxicity[139]. In mice, the sirtuin inhibitor nicotinamide slows the onset of motor phenotypes and corrects some aspects of transcriptional dysregulation in HD model mice, despite not affecting HTT aggregate formation[140]. A clinical trial in HD patients has been conducted with the broad spectrum HDAC inhibitor phenylbutyrate but results have not yet been reported (Steven Hersch, personal communication). Future trials with more specific agents are planned.

In summary, nucleotide repeat elements that cause human disease elicit epigenetic alterations in *cis* and in *trans* that directly impact on the molecular pathogenesis of these disorders. With an ever greater understanding of these alterations and more specific pharmacological interventions possible, epigenetic targets hold great promise for therapeutic development in this patient population.

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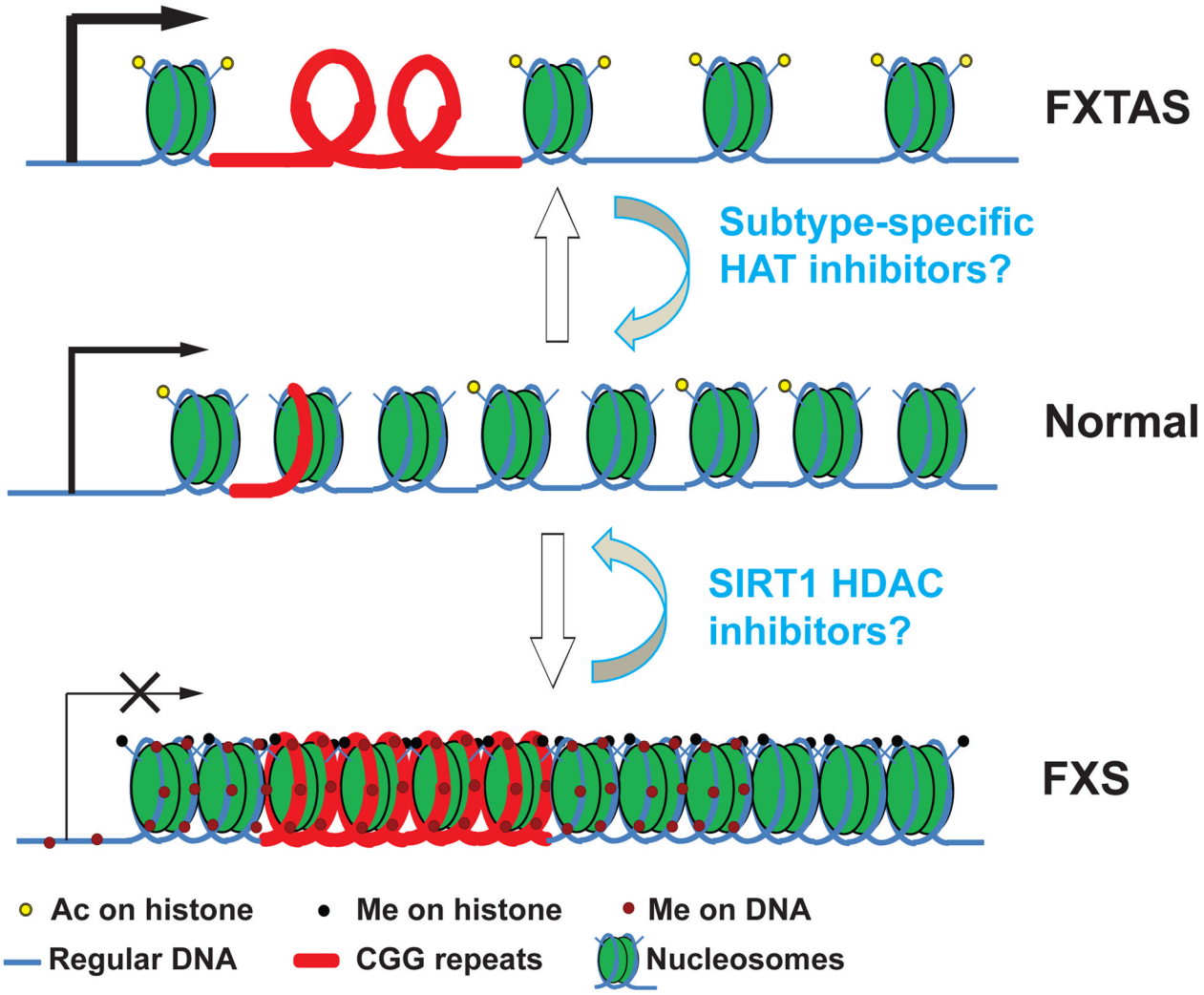

Figure 1. Fragile X Spectrum Disorders: a case of epigenetic extremes

Multiple disorders result from CGG expansions in the 5′UTR of *FMR1*. Middle panel: Normally, there are up to 45 CGG repeats (red segment) in the *FMR1* 5′UTR. These are unmethylated and associated with moderately acetylated histones (yellow dots) and active gene transcription (black arrow). Top panel: in FXTAS, premutation CGG repeat lengths (55–200) are accompanied by hyperacetylation of histones and a more open chromatin state, leading to elevated FMR1 transcription. Bottom panel: in FXS, large (>200 CGG) repeat expansions trigger heterochromatin formation and DNA methylation (dark red dots) across the promoter and through the repeat. Recent studies suggest that histone deacetylation and trimethylation at critical residues such as H3K9 and H3K27 (black dots) precede DNA methylation. However, the mechanism by which this silencing is triggered remains unknown. The combination of heterochromatin formation and DNA methylation silences FMR1 transcription. Potential therapeutic strategies (shown in blue) include use of SIRT1 HDAC inhibitors targeted at reactivation of transcription in FXS and use of subtype-specific HAT inhibitors to suppress excess transcription in FXTAS.

Table 1

Epigenetic alterations surrounding non-coding repeat expansions

Disease	Gene locus	Repeat type	location	Epigenetic changes	Effects on transcription and nearby genes
Myotonic Dystrophy type I (DM1)	DMPK	CTG	3′-UTR	DNA methylation[60,87] Altered CTCF binding	Altered *Six5* expression[80]
DM2	ZNF9	CCTG	Intron 1	Unknown	Decreased ZNF9 expression[141]
FRA12A.MR	DIP2B	CGG	5′-UTR	DNA methylation [142]	Decreased DIP2B expression[142]
FRAXE MR	FMR2	CCG	5′-UTR	DNA methylation[143–144]	Decreased FMR2 expression[143–144]
Friedreich's Ataxia	FXN	GAA	Intron 1	Hypoacetylation and histone methylation [145]	Decreased FXN transcription[145]
Fragile X Syndrome	FMR1	CGG	5′-UTR	Histone methylation and hypoacetylation DNAmethylation [32,44,46]	Decreased or absent FMR1 transcription[32] Absent ASFMR1 and FMR4[59]
FXTAS	FMR1	CGG	5′-UTR	Hyperacetylation of histones[68]	Increased FMR1 transcription[146] Increased ASFMR1 and FMR4[59]
HDL2	JPH3	CTG	3′-UTR	Unknown	Unknown
SCA8	KLH1	CTG	5′-UTR	Unknown	Unknown
SCA10	E46L	ATTCT	3′-UTR	Unknown	Unknown
SCA12	PPP2R2B	CAG	5′-UTR and promoter	Unknown	Increased PPP2R2B transcription[147]
SCA31	BEAN, TK2	TGGAA	Intron	Unknown	Unknown

Table 2

Polyglutamine proteins in epigenetic and transcriptional regulation

Disease	polyQ-proteins	Functions related to transcription/epigenetics	Other cellular functions
DRPLA	Atrophin-1	Transcriptional repressor[148]	
Huntington Disease	Huntingtin	TF interacting protein[120] DNA binding protein[122] CBP interacting protein[112,114,129]	Vesicle Transport Signal Transduction
SBMA	Androgen receptor	Nuclear receptor[149] and transcription factor	
SCA1	Ataxin-1	Transcriptional regulation, histone acetylation[95–97]	
SCA2	Ataxin-2	Transcriptional co-activator[150]	RNA binding[151]
SCA3	Ataxin-3	Transcriptional repressor[152]	Deubiquitinase[153]
SCA6	CACNA1A	C-terminal PolyQ fragment traffics to nucleus[154]	P/Q type Calcium channel[155]
SCA7	Ataxin-7	Coactivator for STAGA histone acetylation complex[102,104]	
SCA17	TBP	General transcription factor[156]	

Abbreviations: DRPLA- Dentatorubral pallidoluysian atrophy; TF- transcription factor; CBP- CREB binding protein; SBMA- Spinal Bulbar Muscular Atrophy (a.k.a. Kennedy's Disease); SCA-Spinocerebellar Ataxiaf; PolyQ- polyglutamine; STAGA- SPT3-TAFII31-GCN5L acetylase complex; TBP- TATA binding protein